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Infradian Biorhythms of Enzymuria in Man?

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Summary: The temporal courses of dipeptidyl peptidase IV γ-glutamyltransferase and alanine aminopeptidase were followed over 70 days in the morning urine of 15 healthy persons. Subsequent to basic statistical analysis a two-step procedure was performed, including spectral analysis and the fit of a cosine function by non-linear regression. The excretion of the 3 enzymes followed an infradian biorhythm with a mean period length of 10.04 for dipeptidyl peptidase IV, 13.34 for γ-glutamyltransferase and 10.17 for alanine aminopeptidase. In addition to the basic rhythmic process described by the fitted cosine functions, in most of the enzyme patterns steap peaks of very high excretory activity appeared which was verified in repeated measurements.

These infradian biorhythms with changes in the range of 100% and more, as well as their interindividual variations, have to be considered in assessing the excretion of enzymes.

Introduction

Urine enzymes are now widely accepted as sensitive indicators for the early detection of renal alterations (see l.c. (1, 2) for references). Since *Grötsch* et al. (3) reported, for the rat, the existence of biological rhythms in the excretion of γ-glutamyltransferase, N-acetyl-β-*D*-glucosaminidase and alanine aminopeptidase with dominant periods in the infradian range of 7 and 9 days, respectively, it has been obvious, that beyond the well described circadian rhythms (4, 5) enzymuria can also be subjected to infradian rhythmic changes.

Therefore, our study was designed to clarify whether or not such infradian changes are also present in man. The urinary excretion of γ-glutamyltransferase (EC 2.3.2.2), alanine aminopeptidase (EC 3.4.11.2) and dipeptidylpeptidase IV (EC 3.4.14.5) was followed for 70 days in 15 volunteers of both sexes, 26 to 55 years old. The detection of relevant periodicities in the individual patterns of all volunteers was ensured by applying spectral analysis and non-linear regression.

Materials and Methods

The urinary enzyme excretion was determined once daily on 70 consecutive days in 15 healthy adults (8 women, 7 men, mean age 39.4, range 26–55 years). Urines were collected between 5 and 9 a.m. and immediately centrifuged (15 min, 1800 *g*). Subsequent to centrifugation the samples were filtered on Sephadex G 50 (medium) with 154 mmol/l NaCl solution (6). The fraction with a relative molecular mass $M_r > 10\,000$ was stored at −20 °C after adding sodium azide solution (15 mmol/l).

γ-Glutamyltransferase activity was pursued at 37 °C for 5 min, using a continuous method. The reaction mixture contained 4.0 mmol/l glutamyl-4-nitroanilide and 101 mmol/l glycylglycine buffer pH 8.20 (37 °C). Spectrophotometric readings were taken at 405 nm.

Alanine aminopeptidase activity was accessed at 37 °C by monitoring the increase in absorbance at 405 nm over 5 min. The final concentrations in the reaction mixture were: 2 mmol/l alanine-4-nitroanilide in 50 µmol/l Tris, pH 7.80 (7).

Dipeptidyl peptidase IV activity was measured by a continuous method using glycylproline-4-nitroanilide as substrate (0.5 mmol/l) in 100 mmol/l tricine-buffer pH 7.40. The absorbance was determined at 405 nm after 2 min incubation at 37 °C (8).

Results were expressed as enzyme activity (kat) per mol urinary creatinine. Creatinine was assessed by a kinetic method.

Subsequent to basic statistics a two-step procedure was applied to determine the rhythmic components of the time series:

1. The original data were subjected to a spectral analysis as described in detail in l.c. (9).
   A 'peak' in the power spectrum (plot of spectral density versus period) indicates that harmonic components of the 'peak' period length provide a prominent contribution to the total energy of the whole time series. Therefore, only these periods are relevant and should be considered. The sharpness of such a peak depends strongly on the length of the time series, and it is reduced by sampling error, the intrinsic biological variability and by dynamic changes that might occur in the biological rhythms.
2. The peak period lengths from spectral analysis served as initial values for the fit of a cosine function to the original data by non-linear regression. As a result estimates of mesor (M), amplitude (A), period (T) and phase (Φ) 95% confidence intervals were obtained.

Results

In addition to random variations, the individual patterns of all three enzymes display regularly occurring maxima of enzymuria. Also, most of the patterns show steap peaks of a very high excretory activity. As an example, the dipeptidyl peptidase IV-, γ-glutamyltransferase- and alanine aminopeptidase-values of volunteer 1 are plotted in figures 1–3. As proved by repeated measurements, these prominent peaks are not caused by errors in the determination of enzyme activity. The plot of all data as individual chronograms allowed a rough assessment of whether the time series were convenient for the application of the mathematical time series analysis.

The results of the statistical analysis provided a basic characterization of the data. As compiled in tables 1–3, the time patterns display distinct individual differences with respect to mean and variability. In some individuals a tendency to high (3, 12, 13) and low (1, 7, 8) coefficients of variation, respectively, is obvious. The generally high variability corresponds to the supposed presence of periodic components in the time series, which, however, must be verified by spectral analysis.

The results of the spectral analysis (depicted as power spectra in figure 4 for volunteer 1) allow a more detailed assessment by revealing the dominant harmonic components of the time series. The comparison

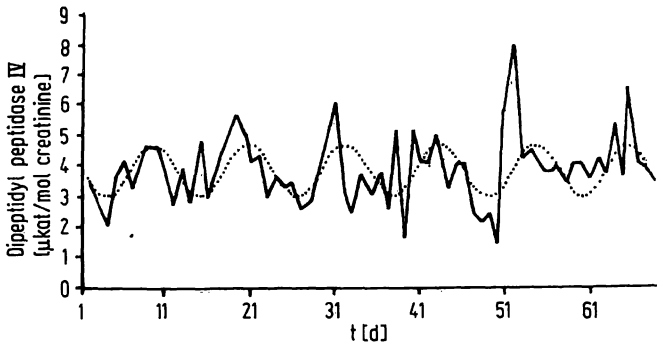


Fig. 1. Graphical demonstration of the urine excretion of dipeptidyl peptidase IV in volunteer 1. The dotted line represents the best fitting cosine function.

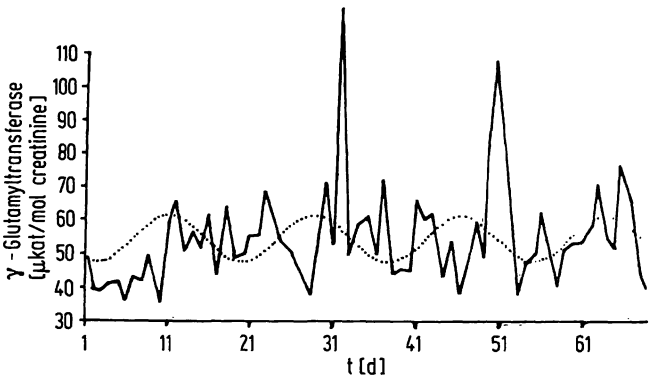


Fig. 2. Graphical demonstration of γ-glutamyltransferase in volunteer 1. The dotted line represents the best fitting cosine function.

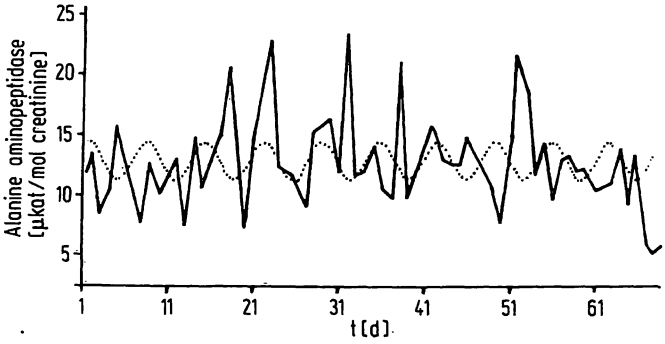


Fig. 3. Graphical demonstration of the urine excretion of alanine aminopeptidase in volunteer 1. The dotted line represents the best fitting cosine function.

of the results of both applied mathematical methods demonstrated that the higher the energy density of the dominant components in the power spectrum, the greater their relevance for the time series and the better these periods correspond to the results of the subsequent non-linear regression.

Thus, the period of 11.3 days clearly dominating in the power spectrum of the dipeptidyl peptidase IV data of volunteer 1 corresponds exactly with the pe-

Tab. 1. Mean, standard deviation, coefficient of variation, and the minimal (Min) and maximal (Max) values of the excretion patterns of dipeptidyl peptidase IV. Except for the coefficient of variation, all data are given in μkat/mol creatinine.

Volunteer No.	Mean	Standard deviation	Coefficient of variation (%)	Min	Max
1	3.8	1.1	30.1	1.3	7.8
2	5.7	2.5	44.3	2.1	16.7
3	8.5	4.5	53.2	1.3	23.7
4	4.8	1.8	36.7	1.4	10.0
5	4.2	1.3	32.3	2.1	9.8
6	4.8	1.8	37.5	2.0	12.3
7	5.3	1.9	36.3	0.9	10.7
8	5.9	2.0	35.0	1.4	14.5
9	4.4	1.9	46.1	1.3	10.3
10	5.5	2.9	51.7	1.6	19.7
11	4.5	2.8	62.6	1.2	21.1
12	3.8	2.1	54.6	1.0	14.5
13	6.4	3.5	55.3	1.3	24.0
14	4.1	1.8	44.7	1.7	8.8
15	5.0	1.5	30.7	1.6	7.8

Tab. 2. Mean, standard deviation, coefficient of variation, and the minimal (Min) and maximal (Max) values of the excretion patterns of γ-glutamyltransferase. Except for the coefficient of variation, all data are given in μkat/mol creatinine.

Volunteer No.	Mean	Standard deviation	Coefficient of variation (%)	Min	Max
1	52.2	15.0	28.7	32.2	120.6
2	84.8	38.2	45.0	38.3	255.5
3	86.2	40.0	46.4	6.2	268.4
4	55.0	24.2	43.9	27.6	207.9
5	67.2	20.3	30.2	20.6	149.1
6	73.1	23.4	32.0	30.4	160.9
7	72.7	18.8	25.9	20.1	134.6
8	82.6	20.5	24.9	33.6	159.7
9	47.1	12.8	27.2	10.6	82.4
10	62.5	29.1	46.5	25.9	220.9
11	57.5	23.0	39.9	36.0	163.4
12	53.2	33.2	62.4	24.7	286.1
13	65.7	28.7	43.7	23.1	221.2
14	54.7	18.9	34.6	18.5	159.9
15	62.2	19.7	31.7	22.2	170.6

riod of 11.22 ± 0.39 calculated by non-linear regression, whereas the coincidence between the flat peaks in the power spectra of γ-glutamyltransferase and alanine aminopeptidase and the respective estimates of non-linear regression is lower (table 4–6). In cases such as the latter two, one should be cautious in interpreting the peaks in the power spectra. However, in most cases the spectral analysis yielded clearly dominant periodic components and provided suitable start values for the following regression.

Tab. 3. Mean, standard deviation, coefficient of variation, and the minimal (Min) and maximal (Max) values of the excretion patterns of alanine aminopeptidase. Except for the coefficient of variation, all data are given in μkat/mol creatinine.

Volunteer No.	Mean	Standard deviation	Coefficient of variation (%)	Min	Max
1	12.3	3.7	29.9	4.7	22.7
2	15.9	6.2	39.2	6.4	41.0
3	35.9	1.7	46.1	13.4	11.6
4	16.0	5.8	36.3	7.1	48.5
5	17.0	7.0	41.3	3.3	57.6
6	22.5	7.2	32.0	9.1	49.7
7	14.8	3.8	25.8	2.2	28.3
8	27.9	9.7	34.6	8.8	68.2
9	14.2	3.3	23.3	5.1	25.8
10	22.3	10.1	45.1	4.1	46.3
11	10.2	3.7	36.3	3.8	29.7
12	14.8	8.6	57.8	5.6	74.8
13	22.0	1.3	59.4	5.5	85.1
14	11.9	4.3	36.1	5.9	29.0
15	16.5	5.7	34.6	5.3	47.5

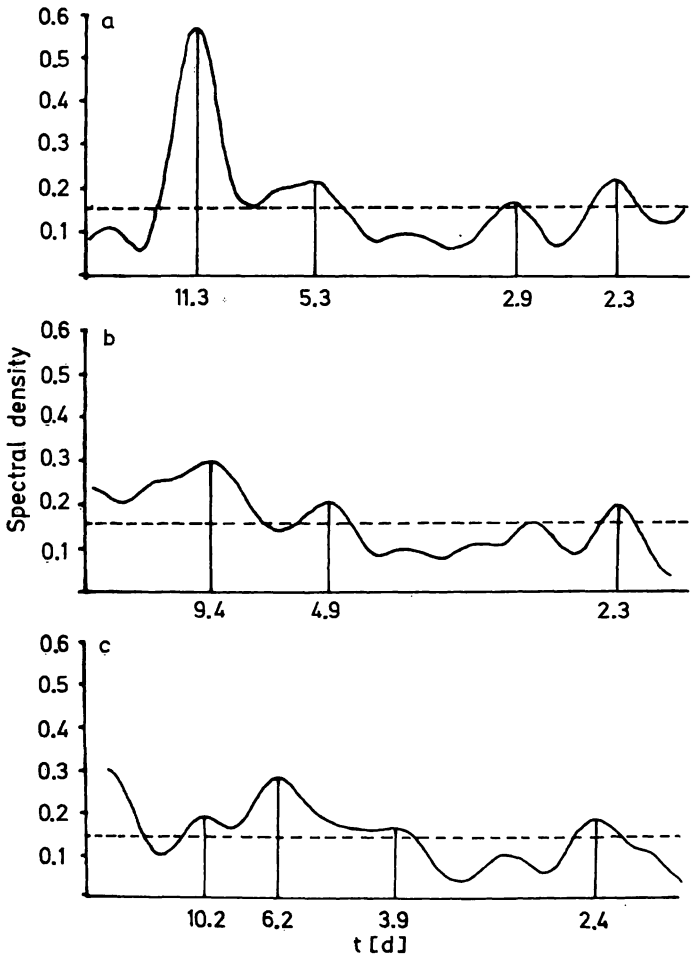


Fig. 4. Power spectra of the excretion patterns of volunteer 1.
a) Dipeptidyl peptidase IV
b) γ-Glutamyltransferase
c) Alanine aminopeptidase
The dotted line represents the energy density of random variations (with noise).
abscissa: period length (tau per day)

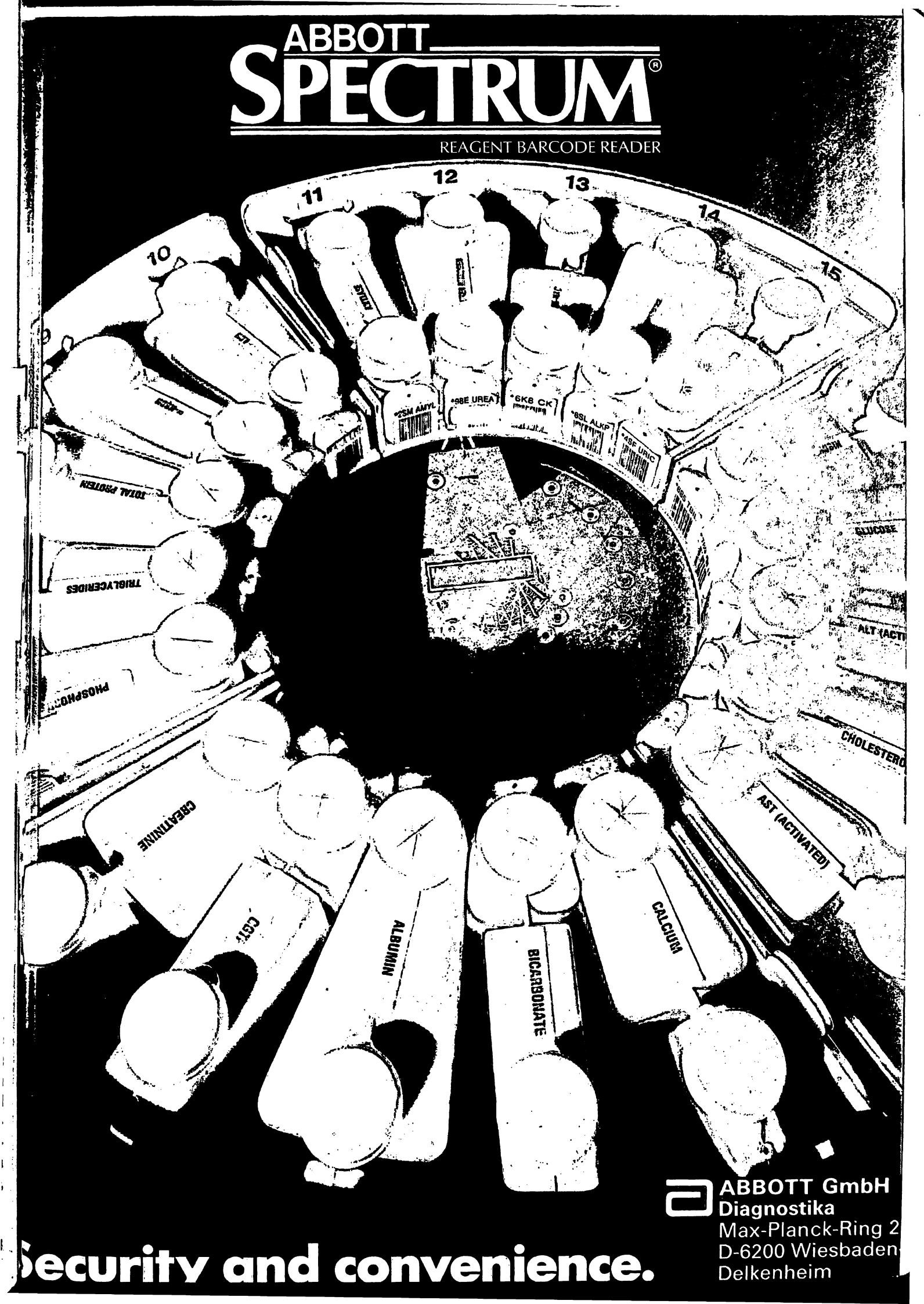
Tab. 4. Results of non-linear regressions used for fitting cosine functions to the excretion patterns of dipeptidyl peptidase IV. Mesor (M), amplitude (A), period (T), phase (Φ) and the respective 95% confidence limits of the best fitting cosine functions are given.
M in μkat/mol creatinine
A in μkat/mol creatinine
T in days, Φ in days

n	T	A	M	Φ
1	11.22 ± 0.39	0.84 ± 0.33	3.78 ± 0.24	8.35 ± 1.09
2	30.25 ± 3.17	1.72 ± 0.79	5.69 ± 0.57	11.09 ± 4.06
3	25.87 ± 2.99	2.52 ± 1.42	8.56 ± 1.05	3.21 ± 5.01
4	13.70 ± 0.85	1.21 ± 0.63	5.02 ± 0.46	5.50 ± 2.38
5	7.16 ± 0.26	0.80 ± 0.46	4.11 ± 0.33	4.69 ± 1.33
6	3.46 ± 0.06	1.04 ± 0.58	4.79 ± 0.41	0.01 ± 0.61
7	7.62 ± 0.32	0.99 ± 0.63	5.34 ± 0.45	5.80 ± 1.62
8	7.11 ± 0.31	1.99 ± 0.70	5.87 ± 0.50	0.66 ± 1.61
9	3.83 ± 0.09	1.13 ± 0.67	4.39 ± 0.48	1.14 ± 0.76
10	16.53 ± 1.53	1.32 ± 0.92	5.45 ± 0.68	5.16 ± 3.69
11	18.84 ± 2.18	1.25 ± 0.98	4.44 ± 0.69	4.39 ± 4.76
12	3.90 ± 0.11	0.97 ± 0.75	3.82 ± 0.53	1.81 ± 0.95
13	5.29 ± 0.19	1.71 ± 1.24	6.41 ± 0.88	2.11 ± 1.29
14	6.79 ± 0.79	0.93 ± 0.67	4.15 ± 0.47	6.67 ± 1.54
15	25.01 ± 3.45	0.88 ± 0.55	5.01 ± 0.39	11.23 ± 4.68

Tab. 5. Results of non-linear regressions used for fitting cosine functions to the excretion patterns of γ-glutamyltransferase. Mesor (M), amplitude (A), period (T), phase (Φ) and the respective 95% confidence limits of the best fitting cosine functions are given.
M in μkat/mol creatinine
A in μkat/mol creatinine
T in days, Φ in days

n	T	A	M	Φ
1	17.63 ± 1.77	6.79 ± 4.86	52.26 ± 3.51	11.06 ± 3.99
2	10.44 ± 0.52	18.44 ± 8.82	83.94 ± 12.67	9.00 ± 2.16
3	n. s.			
4	7.09 ± 0.42	10.86 ± 8.71	54.19 ± 6.11	0.89 ± 1.84
5	20.15 ± 1.89	10.52 ± 6.67	67.53 ± 4.64	18.57 ± 3.83
6	44.38 ± 7.24	15.38 ± 8.71	75.79 ± 5.43	32.15 ± 6.65
7	3.09 ± 0.04	9.39 ± 5.06	6.10 ± 4.30	1.49 ± 0.64
8	22.16 ± 2.06	11.99 ± 6.45	83.03 ± 4.52	16.22 ± 3.82
9	3.85 ± 0.10	4.98 ± 4.30	47.06 ± 3.05	1.10 ± 1.09
10	42.15 ± 14.00	12.33 ± 9.16	62.78 ± 6.90	27.71 ± 11.87
11	21.02 ± 2.50	9.95 ± 7.80	56.45 ± 5.43	20.49 ± 4.91
12	2.00 ± 0.30	36.20 ± 16.40	53.17 ± 7.81	1.42 ± 0.03
13	6.98 ± 0.31	1.99 ± 9.95	63.10 ± 7.01	6.12 ± 1.79
14	9.53 ± 0.48	8.94 ± 6.22	54.52 ± 4.41	3.61 ± 2.11
15	36.19 ± 8.21	9.28 ± 6.45	62.56 ± 4.75	29.34 ± 8.06

The results compiled in tables 4–6 indicate a remarkable variability of the calculated periods with a range of 2 to 44 days. The calculated averages for the investigated sample are in the same range (x ± s); dipeptidyl peptidase IV: 10.04 ± 6.67; γ-glutamyltransferase: 13.34 ± 10.22; alanine aminopeptidase: 10.17 ± 6.68. The results of the spectral analysis and multiple regression, together with the dipeptidyl peptidase IV patterns, seem to be more suited for a cosine-fit than the γ-glutamyltransferase and alanine aminopeptidase data. In one case, the γ-glutamyltransferase excretion pattern of volunteer 3 (table 5), the fit of a cosine wave did not result in a significant amplitude. Four of the volunteers (5, 7, 8, 14) displayed a clear circaseptan (7 ± 1 days) rhythm in the dipeptidyl peptidase IV excretion, but only two in the excretion of γ-glutamyltransferase (4, 13) and alanine aminopeptidase (1, 8). The dominant period length can be very different for the enzymuria of dipeptidyl peptidase IV, γ-glutamyltransferase and alanine aminopeptidase in one and the same person (e. g. volunteers 2, 6). On the other hand, in a few individuals, a general tendency to short (11) and long periods (15), respectively, is evident.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Tab. 6. Results of non-linear regressions used for fitting cosine functions to the excretion patterns of alanine aminopeptidase. Mesor (M), amplitude (A), period (T), phase (Φ) and the respective 95% confidence limits of the best fitting cosine functions are given.
M in μkat/mol creatinine
A in μkat/mol creatinine
T in days, Φ in days

n	T	A	M	Φ
1	6.78 ± 0.30	1.56 ± 1.21	12.33 ± 0.87	0.83 ± 1.74
2	40.88 ± 7.75	3.35 ± 2.22	16.37 ± 1.68	33.46 ± 7.37
3	15.06 ± 1.08	8.59 ± 5.38	36.26 ± 3.92	4.80 ± 2.91
4	4.04 ± 0.08	2.93 ± 1.96	16.03 ± 1.38	4.00 ± 2.91
5	22.55 ± 2.99	3.10 ± 2.29	17.07 ± 1.71	16.41 ± 5.51
6	8.63 ± 0.38	3.65 ± 2.27	22.45 ± 1.63	2.00 ± 1.79
7	5.09 ± 0.19	1.45 ± 1.28	14.87 ± 0.91	4.98 ± 1.43
8	6.06 ± 0.22	4.40 ± 3.10	27.93 ± 2.21	4.33 ± 1.42
9	18.52 ± 2.07	1.40 ± 1.12	14.27 ± 0.79	15.81 ± 4.49
10	17.01 ± 1.50	4.84 ± 3.45	22.53 ± 2.37	6.02 ± 3.52
11	14.49 ± 0.95	2.13 ± 1.16	10.22 ± 8.55	8.03 ± 2.60
12	4.10 ± 0.14	3.25 ± 2.89	14.82 ± 2.05	2.40 ± 1.18
13	4.70 ± 0.15	5.00 ± 4.55	21.94 ± 3.20	1.96 ± 1.32
14	4.12 ± 0.09	1.95 ± 1.40	11.86 ± 0.99	3.15 ± 0.96
15	32.35 ± 9.28	2.52 ± 1.86	16.58 ± 1.37	24.32 ± 8.94

Discussion

The presented results demonstrate that enzymuria undergoes significant infradian changes in man. Hence, rhythms in enzymuria are not restricted to one frequency domain, but they cover at least a range from periods of a few hours (3) to 44 days as shown here.

The variability in the period length of all 3 enzymes confirms the experience that in man rhythmicity is very often rather an individual than an interindividually synchronized event. Whereas for most mammals light is the main 'zeitgeber', in man the social environment acts as the most powerful entraining agent (10). In that our volunteers were not hospitalized their social regime and, hence, the phase of their 'zeitgeber' was different. Therefore, it is not surprising that animals with a nearly indentical genotype, housed under the same lighting conditions show a good phase coincidence, as observed in Wistar rats (3), but human beings living under different social conditions fail to show the same interindividual synchronization.

The chosen mathematical two-step procedure ensures high reliability of the results. The disadvantage of the relatively short time series for performing spectral analysis as well as the insensitivity of spectral analysis to slight trends in the time series could be overcome by applying the non-linear regression as a final step. However, it must be emphasized, that although time series analysis is a very useful tool in analysing biological data in time, one should be cautious in interpreting the results of such procedures.

From the parallel plot of the original data and the calculated cosine only one part of the time series' periodic component can be described by a cosine function. Steep peaks of enormous excretory activity regularly appearing in most of the excretion patterns seem to overlay the basis rhythmic process, featured by the fitted cosine wave. Therefore, it can be deduced that periodicity in enzymuria represents a complex phenomenon perhaps regulated by different internal pacemakers. The kidneys appear to contain their own pacemakers which are under control of 'master oscillators' in the CNS (11, 12). However, at present the concrete genesis of the rhythms in enzymuria is still a matter of speculation.

Urine enzyme catalytic activities have been known to be affected by exogenous pharmacological (13, 14) and endogenous circadian, sex-specific, hormonal and genetic (4, 5, 15–17) influences. The elaborated infradian changes add a new systematic factor to this spectrum. But, as long as the biological mechanisms underlying such changes are not clear the changes themselves are not predictable. Thus, they appear as biological noise and make it difficult to discriminate between normal and pathological conditions.

What Are the Implications of our Findings for the Clinic?

The described infradian rhythms in enzymuria exhibit changes of 100% and more. Therefore, it is recommendable to gather several urine samples of one patient on consecutive days and to use the mean (after

removing outliers) or the median (the value in the middle of all measurements ordered according to size) as a basis for diagnosis.

In this context the question arises of whether it is possible to optimize the effect and to minimize side-effects of nephrotropic drugs in applying them at certain stages of the infradian rhythms. In the case of circadian rhythms this principle was used by *Levi* et al. (18) to minimize the nephrotoxic effects of *cis*-diamminedichloroplatinum in rats. This problem as well as the examination of an age-dependency of the rhythms in enzyme excretion require further investigation.

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